FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
1038-1030 MIS:jb	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	
09/554333	
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE
PCT/CA98/01065	13 November 1998
PRIORITY DATE CLAIMED	
14 November 1997	

TITLE OF INVENTION
ALPHAVIRUS VECTORS

APPLICANT(S) FOR DO/EO/US
Mark Parrington; and Michel H. Klein

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). - unsigned copy
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ Certificate of Mailing by Express Mail
20. ☐ Other items or information:

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/554333	PCT/CA98/01065	1038-1030 MIS:jb

21. The following fees are submitted:.	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :	
☒ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO **$840.00**	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$690.00**	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$970.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	14 - 20 =	0	x $18 00	**$0.00**
Independent claims	2 - 3 =	0	x $78 00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**

TOTAL OF ABOVE CALCULATIONS =	**$970.00**
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).** ☐	**$0.00**
SUBTOTAL =	**$970.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +	**$0.00**
TOTAL NATIONAL FEE =	**$970.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐	**$0.00**
TOTAL FEES ENCLOSED =	**$970.00**
Amount to be: refunded	$
charged	$

☒ A check in the amount of **$970.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **19-2253** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Mr. Michael I. Stewart
Sim & McBurney
6th Floor, 330 University Avenue
Toronto, Ontario
Canada, M5G 1R7

SIGNATURE

Michael I. Stewart
NAME

24,973
REGISTRATION NUMBER

May 11, 2000
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Our Ref: 1038-1030 MIS:jb

In re National Phase of International Application No.: PCT/CA98/01065

International Filing Date: 13 November 1998

Applicant: Mark Parrington, et al.

Title: ALPHAVIRUS VECTORS

PRELIMINARY AMENDMENT

The Commissioner of Patents
and Trademarks,
Washington, D.C. 20231,
U. S. A.

Dear Sir:

Please amend this application in the following manner:

In the Disclosure:

Before the first line of the specification, add the following:

" REFERENCE TO RELATED APPLICATIONS

This application is a national phase application under 35 U.S.C. 371 of PCT/CA98/01065."

REMARKS

The specification has been amended on page 1 to reflect that this application is a U.S. National Phase filing under 35 U.S.C. 371 of PCT/CA98/01065.

Respectfully submitted,

Michael I. Stewart
Reg. No. 24,973

Toronto, Ontario, Canada
(416) 595-1155
FAX No. (416) 595-1163

Date: May 11, 2000

TITLE OF INVENTION

ALPHAVIRUS VECTORS

FIELD OF INVENTION

The present invention relates to the field of DNA vaccines and is particularly concerned with modified alpha virus vectors for use in such vaccines.

BACKGROUND OF THE INVENTION

Semliki Forest virus (SFV) is a member of the Alphavirus genus in the Togaviridae family. The mature virus particle contains a single copy of a ssRNA genome with a positive polarity that is 5'-capped and 3'-polyadenylated. It functions as an mRNA and naked RNA can start an infection when introduced into cells. Upon infection/transfection, the 5' two-thirds of the genome is translated into a polyprotein that is processed into the four nonstructural proteins (nsP1 to 4) by self cleavage. Once the ns proteins have been synthesized they are responsible for replicating the plus-strand (42S) genome into full-length minus strands (ref. 14). These minus-strands then serve as templates for the synthesis of new plus-strand (42S) genomes and the 26S subgenomic mRNA (ref. 1 - Throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification. The disclosures of these references are hereby incorporated by reference into the present disclosure). This subgenomic mRNA, which is colinear with the last one-third of the genome, encodes the SFV structural

proteins. In 1991 Liljestrom and Garoff (ref. 2) designed a series of expression vectors based on the SFV CDNA replicon. These vectors had the virus structural protein genes deleted to make the way for heterologous inserts, but preserved the nonstructural coding region for production of the nsP1 to 4 replicase complex. Short 5' and 3' sequence elements required for RNA replication were also preserved. A polylinker site was inserted downstream from the 26S promoter followed by translation stop sites in all three frames. An SpeI site was inserted just after the 3' end of the SFV CDNA for linearization of the plasmid for use *in vitro* transcription reactions.

Injection of SFV RNA encoding a heterologous protein have been shown to result in the expression of the foreign protein and the induction of antibody in a number of studies (refs. 3,4). The use of SFV RNA inoculation to express foreign proteins for the purpose of immunization would have several of the advantages associated with plasmid DNA immunization. For example, SFV RNA encoding a viral antigen may be introduced in the presence of antibody to that virus without a loss in potency due to neutralization by antibodies to the virus. Also, because the protein is expressed *in vivo* the protein should have the same conformation as the protein expressed by the virus itself. Therefore, concerns about conformational changes which could occur during protein purification leading to a loss in immunogenicity, protective epitopes and possibly immunopotentiation, could be avoided by plasmid DNA immunization.

In WO95/27044, the disclosure of which is incorporated herein by reference, there is described the use of alphavirus cDNA vectors based on cDNA complementary to the alphavirus RNA sequence. Once transcribed from the cDNA under transceptional control of a heterologous promoter, the alphavirus RNA is able to self-replicate by means of its own replicase and thereby amplify the copy number of the transcribed recombinant RNA molecules.

SUMMARY OF THE INVENTION

The present invention is concerned with modifications to the alphavirus cDNA vectors described in the aforementioned WO 95/27044 to permit enhanced replication of the alphavirus. In the present invention, a heterologous splice site is introduced into the alphavirus replicon sequence, particularly that of Semliki Forest virus (SFV).

Accordingly, in one aspect, the present invention provides an expression vector comprising a DNA molecule complementary to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions which are essential for replication of the said alphavirus RNA, and further comprises a heterologous DNA sequence capable of expression in a suitable host, such as a human or animal host, said heterologous DNA sequence being inserted into a region of the DNA molecule which is non-essential to replication thereof, and the DNA molecule being placed under transcriptional control of a promoter sequence functional in said animal or human host, wherein at least one heterologous splice site is

provided in the DNA molecule to prevent aberrant RNA splicing of the alphavirus.

The alphavirus molecule is a large molecule and, accordingly, there is a high probability of cryptic splice sites, thereby impairing the replication of the alphavirus and hence its ability to express the heterlogous DNA is impaired. By introducing the at least one optimal heterologous splice site in accordance with the present invention into the alphavirus replicon sequence, any splicing is likely to be directed at the heterologous splice site rather than any cryptic splice sites, restores the function of the SFV replicon when removed, and may improve transport of RNA from the nucleus (ref. 6).

In the constructs provided herein, the promoter is placed upstream of the 5'-end of the alphavirus sequence, such that the resultant transcript has an authentic 5'-end, which is required for the efficient replication of the alphavirus RNA replicon.

In addition, there may be provided at the 3'end of the Semliki Forest virus segment, a hepatitis delta virus ribozyme sequence to ensure proper *in vivo* cleavage at the 3'-end of the sequence. Any other convenient sequence may be employed to achieve this effect.

The heterologous splice site sequence may be provided by the nucleotide sequence of the rabbit β-globin intron II, as described in reference 5. Such heterologous splice site sequence may be inserted into the complement sequence at any convenient location which generates perfect splice junctions. This

precludes replication of the alphavirus, unless it is authentically removed by splicing..

I have identified five suitable sites in the SFV replicon, which are contained within an EcoRV-SpeI fragment of the replicon which is 8010 bp in length (Fig. 3). The first such site is a Ppu-MI site, at position 2719 within the EcoRV-SpeI fragment.

In constructing the modified vectors provided herein, the EcoRV-SpeI fragment is cut with Ppu-MI at position 2719 and made blunt-ended with Mung Bean nuclease, which removes three bases from the SFV sequence. A blunt-ended β-globin II intron, which is 536 bp long, is ligated into the site and replaces the missing three bases with sequence added to the 3'-end of the β-globin intron sequence (Fig. 1).

The other four suitable sites for insertion of the Intron are the PvuII sites at bp 2518, 3113, 6498 and 6872 of the EcoRV-SpeI fragment. Insertion of the Intron is achieved by cutting with PvuII (a blunt end cutter) and the blunt-ended β-globin II intron sequence (Fig. 2) is ligated into one or more of these sites.

In a further aspect of the present invention, there is provided a cloning vector suitable for expression in a host cell of an heterologous DNA sequence, which comprises a DNA molecule complementing to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions and has a cloning site for insertion therein of a heterologous DNA sequence capable of expression in a host cell, said cloning site being located in a region of the DNA molecule which is

non-essential to replication thereof; a promoter sequence functional in said host cell and transcriptionally controlling said DNA molecule, said promoter sequence being placed upstream of the 5'-end of the DNA molecule such that the resultant transcript had an authentic 5' end; at least one heterologous splice set provided in the complement of the DNA molecule to generate perfect splice junctions in the alphavirus in order to prevent aberrant splicing and an additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the reactant mRNA transcript.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows the DNA sequence of the β-globin intron II including three additional nucleotides at the 3'-end thereof (SEQ ID No:1);

Figure 2 shows the DNA sequence of the β-globin intron II (SEQ ID No:2);

Figures 3A to 3C show the DNA sequence of the EcoRV-SpeI fragment of Semliki Forest virus replicon (SEQ ID No:3);

Figures 4A to 4D show the DNA sequence of the pSFV link (SEQ ID no: 4) prepared as illustrated in Figure 5;

Figure 5 shows construction of pSFVlink (11060 bp) from pSFV1 using a linker sequence (SEQ ID nos: 5,6);

Figures 6A to 6D show the nucleotide sequence of plasmid pMP76 (SEQ ID no: 11, prepared as illustrated in Figures 8A to 8D;

Figure 7 illustrates subsections of plasmid pSFV link (see Figure 5);

Figure 8A to 8D show the construction of plasmid pMP76 from plasmids pMP53, pMP70, pMP47, pMP55 and pMP71;

Figures 9A to 9B show the construction of plasmids pMP53, pMP54 and pMP55 from plasmid pMP52;

Figure 10 shows the construction of plasmid MP52 from pUC19 using a linker sequence (SEQ ID no: 7,8);

Figures 11A to 11B show the construction of plasmids pMP46, pMP47 and pMP70 from pUC19 and fragment from pSFV link, prepared as seen in Figure 7; and

Figures 12A to 12B show the construction of plasmid pMP71 from plasmid pCMV3.

GENERAL DESCRIPTION OF INVENTION

As discussed above, the present invention provides a modified alphavirus DNA. The alphavirus preferably is Semliki Forest virus. In particular, the present invention provides a cloning vector for heterologous gene expression in a host, such as an animal or human.

The promoter sequence may comprise a promoter of eukaryotic or prokaryotic origin. Suitable promoters are the cytomegalovirus immediate early promoter (pCMV), although other promoters, such as the Rous sarcoma virus long-terminal repeat promoter (pRSV), since, in the case of these and similar promoters, transcription is performed by the DNA-dependent RNA polymerase of the host cell. Additionally, the SP6, T3 or T7 promoters can be used, provided that the cell has first been transformed with genes encoding SP6, T3 or T7 RNA polymerase molecules which are either inserted into the chromosome or remain episomal. Expression of

these (SP6, T3, T7) RNA polymerase-encoding genes is dependent on the host cell DNA-dependent RNA polymerase.

The heterologous DNA insert may comprise the coding sequence for a desired product, which may be a biologically active protein or polypeptide, for example, the heterologous DNA insert may code for HIV sequences, e.g., an immunogenic or antigenic protein or polypeptide, or a therapeutically active protein or polypeptide. The heterologous DNA may also comprise additional sequences, such as a sequence complementary to an RNA sequence which is a self-cleaving ribozyme sequence.

The DNA vectors provided herein may be administered to a host, including a human host, for *in vivo* expression of the heterologous DNA sequence, in accordance with a further aspect of the invention, in order to generate an immune response in the host, which may be a protective immune response. The DNA vectors may be further formulated into immunogenic compositions for such administration.

BIOLOGICAL DEPOSITS

Certain vectors that contain the Semliki Forest virus replicon and referred to herein have been deposited with the American Type Culture Collection (ATCC) located at 10801 University Boulevard, Manassas, VA 20110-2209, U.S.A., pursuant to the Budapest Treaty and prior to the filing of this application.

Samples of the deposited plasmids will become available to the public upon grant of a patent based

upon this United States patent application and all restrictions on access to the deposits will be removed at that time. Non-viable deposits will be replaced. The invention described and claimed herein is not to be limited in scope by plasmids deposited, since the deposited embodiment is intended only as an illustration of the invention.

Deposit Summary

Plasmid	ATCC Designation	Date Deposited
pMP76		

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

EXAMPLE 1

This Example describes the construction of plasmid pMP76 as outlined in Figures 5, 7, 8A, 8B, 8C, 8D, 9A, 9B, 10, 11A, 11B, 12A and 12B.

Plasmid pSFV link was created by restricting plasmid pSFV1 (Gibco) with BamHI. This plasmid was then ligated with a linker (SEQ ID no: 5 and 6) to produce plasmid pSFV link (Figures 4A to 4D, Figure 5).

Some of the SFV replicon fragments were subcloned by restricting pSFVlink with EcoRV and SpeI and isolating the 890bp EcoRV-SpeI fragment. This fragment was then restricted with EcoRI and the 1906bp EcoRV-EcoRI, the 1578bp and 3627bp EcoRI-EcoRI and the 899bp EcoRI-SpeI fragments isolated (Fig.7).

The 1909bp EcoRV-EcoRI SFV fragment was cloned into EcoRV-EcoRI restricted plasmid pMP52 to produce plasmid pMP53 (Fig.9A). The 899bp EcoRI-SpeI SFV fragment was cloned into EcoRI-SpeI restricted pMP52 to produce pMP54 (Fig.9A). Plasmid pMP54 was then restricted with SpeI and made blunt-ended with Mung Bean nuclease. The plasmid was then restricted with BglII, dephosphorylated and ligated to the hepatitis delta virus ribozyme linker (SEQ ID nos. 9 and 10), that had been phosphorylated, to produce pMP55 (Fig. 9B).

Plasmid pMP52 was created by ligating a linker (SEQ ID nos:7,8), into the EcoRI site of pUC19 (Fig.10).

The 1578bp EcoRI-SFV fragment ws cloned into the EcoRI site of pUC19, to produce pMP46 (Fig.11A). This plasmid was then restricted with PpuM1 and made

blunt-ended with Mung Bean nuclease. The rabbit ß-globin intron II PCR fragment (Fig.1) was made blunt-ended with Mung Bean nuclease, phosphorylated and ligated to the PpuMI restricted pMP46 to produce plasmid pMP70 (Fig.11B).

The 3627bp EcoRI SFV fragment was cloned into the EcoRI site of pUC19 to produce pMP47 (Fig.11A).

Plasmid pCMV3, which contains the CMV promoter, Intron A sequence, BGH poly A sequence and SU40 poly A sequence, was restricted with NdeI and EcoRV. The 3191bp NdeI-EcoRV fragment was isolated and dephosphorylated. The 1321bp NdeI-EcoRV fragment was isolated and restricted with SacI. The NdeI-SacI fragment of 334bp was isolated (Fig.12A). The isolated SacI-EcoRV PCR fragment containing the 5'-end of SFV was ligated to the previously isolated 334bp NdeI-SacI fragment and the 3191bp NdeI-EcoRV fragment to produce pMP71 (Fig.12A and 12B).

Plasmid pMP53 was then restricted with EcoRI and BamHI and ligated to the isolated and dephosphorylated 2151bp EcoRI fragment from pMP70 (Fig.8A). This ligation was then restricted with EcoRV and the 4057bp EcoRV-EcoRI fragment purified(Fig.8A).

Plasmid pMP47 was restricted with EcoRI and the 3627bp EcoRI fragment isolated and dephosphorylated (Fig.8B). Plasmid pMP55 was then restricted with BglII, dephosphorylated and restricted with EcoRI. The 985bp EcoRI-BglII fragment was isolated and ligated to the previously isolated EcoRI fragment from pMP47 (Fig.8B). The ligation reaction was then

phosphorylated and the 4612bp EcoRI-BglII fragment isolated.

Plasmid pMP71 was restricted with EcoRV and BamHI then dephosphorylated. This fragment was used in a 3-way ligation with the previously isolated 4612bp EcoRI-BglII fragment from pMP47 and pMP55, and the 4057bp EcoRV-EcoRI fragment from pMP53 and pMP70, to produce pMP76 (Figs.8B and 8C).

The 5' end of the SFV replicon was produced by PCR amplification of pSFV1 using primers SFV-5'-3 having the sequence

5'-ATCTATGAGCTCGTTTAGTGAACCGTATGGCGGATGTGTGACATACA-3'

and EcoR-SPE having the sequence

5'-TCCACCTCCAAGGATATCCAAGATGAGTGTG-3' (SEQ ID no: 9 and SEQ ID no: 10 respectively) between the CMV promoter and the 5' end of the SFV replicon. The resulting PCR fragment was restricted with SacI and EcoRV (Fig. 13; SEQ ID no: 11) and the fragment isolated.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides a modified alphavirus-based expression vector wherein at least one optimal splice site is introduced to the alphavirus replicon to prevent aberrant splicing of the alphavirus genome; and improve transport of RNA out of the nucleus. Modifications are possible within the scope of the invention.

REFERENCES

1. Fulginiti, V.A., Eller, J.J., Sieber, O.F., Joyner, J.W., Minamitani, M. and Meiklejohn, G., (1969) Am. J. Epidemiol. 89 (4), 435-448.

2. Chin, J., Magoffin, R.L., Shearer, L.A., Schieble, J.H. and Lennette, E.H. (1969) Am. J. Epidemiol. 89 (4), 449-463.

3. Jensen, K.E., Peeler, B.E. and Dulworth, W.G. (1962) J. Immunol. 89, 216-226.

4. Murphy, B.R., Prince, G.A., Collins, P.L., Van Wyke-Coelingh, K., Olmstead, R.A., Spriggs, M.K., Parrott, R.H., Kim, H.-Y., Brandt, C.D. and Chanock, R.N. (1988) Vir. Res. 11, 1-15.

5. Chapman, B.S.; Thayer, R.M.; Vincent, K.A. and Haigwood, N.L., Nucl. Acids. Res. 1991, 19: 3979-3986.

6. Huang, Zhi-ming and Yen, T. S. Benedict, Molecular and Cell Biology, July 1995, p.3864-3869.

CLAIMS

1. An expression vector, comprising a DNA molecule complementary to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions which are essential for replication of the said alphavirus RNA and further comprises a heterologous DNA seuence capable of expression in a host, said heterologous DNA sequence being inserted into a region of the DNA molecule which is non-essential to replication thereof, and the DNA molecule being placed under transcriptional control of a promoter sequence functional in said host, wherein at least one heterologous splice site is provided in the DNA molecule to prevent aberrant RNA splicing of the alphavirus.

2. The vector of claim 1 wherein said promoter is placed upstream of the 5'-end of the DNA molecule such that the resultant transcript has an authentic 5'-end.

3. The vector of claim 2 wherein said promoter is the cytomegalovirus immediate early promoter.

4. The vector of claim 1 which further comprises an additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the DNA molecule.

5. The vector of claim 4 wherein said additional DNA sequence comprises a hepatitis delta ribozyme sequence.

6. The vector of claim 1 wherein the heterologous splice site sequence is provided by the DNA sequence of the rabbit ß-globin intron II.

7. The vector of claim 6 wherein the heterologous splice site sequence is inserted into the DNA molecule

at a location which generates perfect splice junctions and restores the function of the SFV replicon when removed.

8. The vector of claim 1 wherein the alphavirus is a Simliki Forest virus.

9. A cloning vector suitable for expression in a host cell of an heterologous DNA sequence, which comprises:

a DNA molecule complementing to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions and has a cloning site for insertion therein of a heterologous DNA sequence capable of expression in a host cell, said cloning site being located in a region of the DNA molecule which is non-essential to replication thereof;

a promoter sequence functional in said host cell and transcriptionally controlling said DNA molecule, said promoter sequence being placed upstream of the 5'-end of the DNA molecule such that the resultant transcript had an authentic 5' end;

at least one heterologous splice set provided in the complement of the DNA molecule to permit aberrant RNA splicing of one to generate perfect splice junctions in the alphavirus; and

an additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the reactant RNA molecule.

10. The cloning vector of claim 9 wherein said heterologous splice set is provided by the DNA sequence of the rabbit ß-globin intron II.

11. The cloning vector of claim 9 wherein said additional sequence comprises a hepatitis delta ribozyme sequence.

12. The cloning vector of claim 8 wherein the alphavirus is a Semliki Forest virus.

13. The cloning vector of claim 8 which has the identifying characteristics of plasmid pMP76 shown in Figure 8D.

14. The cloning vector of claim 8 having SEQ ID no: 11.

FIG.1

Nucleotide Sequence of the β-globin intron II with the 3' SFV bases


```
gtgagtttgg ggacccttga ttgttctttc ttttcgcta ttgtaaaatt catgttatat 60
ggaggggggca aagttttcag ggtgttgttt agaatgggaa gatgtcccct gtatcaccat 120
ggaccctcat gataattttg tttcttttcac ttttctactct gttgacaacc attgtctccct 180
cttatttcct tttcatttttc tgtaactttt tcgttaaact ttagcttgca tttgtaacga 240
attttaaat tcactttgt ttatttgtca gattgtaagt actttctcta atcacttttt 300
ttcaaggca atcagggtat attatattgt acttcagcac agttttagag aacaattgtt 360
ataattaaat gataaggtag aatatttctg catataaatt ctggctggcg tggaaatatt 420
cttattggta gaaacaacta catcctggtc atcatcctgc ctttctcttt atggttacaa 480
tgatatacac tgtttgagat gaggataaaa tactctgagt ccaaaccggg cccctctgct 540
aaccatgttc atgccttctt ctttttccta caggtc 576
```

FIG.2

Nucleotide Sequence of the β-globin intron II

```
gtgagtttgg ggacccttga ttgttctttc ttttcgcta ttgtaaaatt catgttatat 60
ggaggggggca aagttttcag ggtgttgttt agaatgggaa gatgtcccttgtatcaccat 120
ggaccctcat gataattttg tttcttttcac tttctactct gttgacaacc attgtctcct 180
cttattttct tttcatttttc tgtaacttttt tcgttaaact ttagcttgca tttgtaacga 240
attttaaat tcacttttgt ttatttgtca gattgtaagt actttctcta atcacttttt 300
tttcaaggca atcagggtat attatattgt acttcagcac agttttagag aacaattgtt 360
ataattaaat gataaggtag aatatttctg catataaatt ctggctggcg tggaaatatt 420
cttattggta gaaacaacta catcctggtc atcatcctgc ctttctcttt atggttacaa 480
tgatatacac tgtttgagat gaggataaaa tactctgagt ccaaaccggg cccctctgct 540
aaccatgttc atgccttctt cttttcctca cag                              573
```

FIG.3A

Eco RV-SpeI Fragment of Semliki Forest virus replicon

```
atcggcagtg cgccttccag gagaatgatg tctacgcaca aataccactg cgtatgccct 60
atgcgcagcg cagaagaccc cgaaaggctc gatagctacg caaagaaact ggcagcggcc 120
tccgggaagg tgctggatag agagatcgca ggaaaaatca ccgacctgca gaccgtcatg 180
gctacgccag acgctgaatc tcctaccttt tgcctgcata cagacgtcac gtgtcgtacg 240
gcagccgaag tggccgtata ccaggacgtg tatgctgtac atgcaccaac atcgctgtac 300
catcaggcga tgaaaggtgt cagaacggcg tattggattg ggtttgacac cacccgtttt 360
atgtttgacg cgctagcagg cgcgtatcca acctacgcca caaactgggc cgacgagcag 420
gtgttacagg ccaggaacat aggactgtgt gcagcatcct tgactgaggg aagactcggc 480
aaactgtcca ttctccgcaa gaagcaattg aaaccttgcg gctggcactt accctccgta 600
ggatctacat tgtacactga gagcagaaag ctactgagga gtaccatcgt atcatgtgaa 660
ttccacctga aaggtaaaca atcctttacc tgtaggtgcg ataccatcgt acggtaaaac 720
gggtacgtag ttaagaaaat cactatgtgc cccggcctgt acggtaaaac ggtagggtac 780
gccgtgacgt atcacgcgga gggattccta gtgtgcaaga ccacagacac tgtcaaagga 840
gaaagagtct cattcctgt atgcacctac gtccctcaa ccatctgtga tcaaatgact 900
ggcatactag cgaccgacgt cacaccggag gacgcacaga agttgttagt gggattgaat 960
cagaggatag ttgtgaacgg aagaacacag cgaaacacta acagcgatgaa gaacttatctg 1020
cttccgattg tggccgtgcc atttagcaag tgggcgaggg aatacaaggc agaccttgat 1080
gatgaaaaac ctctgggtgt ccgagagagg tcacttactt gctgctgctt gtgggcattt 1140
aaaacgagga agatgcacac catgtacaag aaaccagaca cccagacaat agtgaaggtg 1200
ccttcagagt ttaactcgtt cgtcatcccg agcctatggt ctacaggcct cgcaatccca 1260
gtcagatcac gcattaagat gcttttggcc aagaagacca agcgagagtt aatacctgtt 1320
ctcgacgcgt cgtcagccag ggatgctgaa caagaggaga aggagagtt ggaggccgag 1380
ctgactagag aagccttacc acccctcgtc cccatcgcgc cggcggagac gggagtcgtc 1440
gacgtcgacg ttgaagaact agagtatcac gcaggtgcag gggtcgtgga aacacctcgc 1440
agcgcgttga aagtcaccgc acagccgaac gacgtactac taggaaatta cgtagttctg 1500
```

FIG.3B

```
tccccgcaga ccgtgctcaa gagctccaag ttggcccccg tgcaccctct agcagagcag 1560
gtgaaaataa taacacataa cgggagggcc ggcggttacc aggtcgacgg atatgacggc 1620
agggtcctac taccatgtgg atcggccatt ccggtccctg agtttcaagc ttgagcgag 1680
agcgccacta tggtgtacaa cgaaagggag ttcgtcaaca ggaaactata ccatattgcc 1740
gttccacgga cgtgctgtaa caccgacgag gagaactacg agaaagtcag agctgaaaga 1800
actgacgccg agtacgtgtt cgacgtagat aaaaaatgct gcgtcaagag agaggaagcg 1860
tcgggtttgg tgttggtggg agagctaacc aaccccccgt tccatgaatt cgcctacgaa 1920
gggctgaaga tcaggccgtc ggcaccatat aagactacag tagtaggagt ctttgggttt 1980
ccgggatcag gcaagtctgc tattattaag agcctcgtga ccaaacacga tctggtcacc 2040
agcggcaaga aggagaactg ccaggaaata gttaacgacg tgaagaagca ccgcgggaag 2100
gggacaagta gggaaaacag tgactccatc ctgctaaacg ggtgtcgtcg tgccgtggac 2160
atcctatatg tggacgaggc tttcgcttgc cattccggta ctctgctggc cctaattgct 2220
cttgttaaac ctcggagcaa agtggtgtta tgcggagacc ccaagcaatg cggattcttc 2280
aatatgatgc agcttaaggt gaacttcaac cacaacatct gcactgaagt atgtcataaa 2340
agtatatcca gacgttgcac gcgtccagtc acggccatcg tgtctacgtt gcactacgga 2400
ggcaagatgc gcacgaccaa cccgtgcaac aaacccataa tcatagacac cacaggacag 2460
accaagccca agccaggaga catcgtgtta acatgcttcc gaggctgggc aaagcagctg 2520
cagttggact accgtggaca cgaagtcatg acagcagcag catcttcagg cctcacccgc 2580
aaagggtat acgccgtaag gcagaaggtg aatgaaaatc ccttgtatgc ccctgcgtcg 2640
gagcacgtga atgtactgct gacgcgcact gaggataggc tggtgtggaa aacgctggcc 2700
ggcgatccct ggattaaggt cctatcaaac atccacagg gtaactttac ggccacattg 2760
gaagaatggc aagaagaaca cgacaaaata atgaaggtga ttgaaggacc ggctgcgcct 2820
gtggacgcgt tccagaaaca agcgaacgtg tgttgggcga aaagcctggt gcctgtcctg 2880
gacactgccg gaatcagatt gacagcagag gagtggagca ccataattac agcatttaag 2940
gaggacagag cttactctcc agtggtggcc ttgaatgaaa tttgcaccaa gtactatgga 3000
gttgacctgg acagtggcct gttttctgcc ccgaaggtgt ccctgtatta cgagaacaac 3060
```

FIG.3C

```
cactgggata acagacctgg tggaaggatg tatggattca atgccgcaac agctgccagg 3120
ctggaagcta gacatacctt cctgaagggg cagtggcata cgggcaagca ggcagttatc 3180
gcagaaagaa aaatccaacc gctttctgtg ctggacaatg taattcctat caaccgcagg 3240
ctgccgcacg ccctggtggc tgagtacaag acggttaaag gcagtagggt tgagtggctg 3300
gtcaataaag taagagggta ccacgtcctg ctggtgagtg agtacaacct ggctttgcct 3360
cgacgcaggg tcacttggtt gtccaccgctg aatgtcacag gcgccgatag gtgctacgac 3420
ctaagtttag gactgccggc tgacgccggc aggttcgact tggtctttgt gaacattcac 3480
acggaattca gaatccacca ctaccagcag tgtgtcgacc acgccatgaa gctgcagatg 3540
ctggggggag atgcgctacg actgctaaaa cccggcggca tcttgatgag agcttacgga 3600
tacgccgata aaatcagcga agccgttgtt tcctccttaa gcagaaagtt ctcgtctgca 3660
agagtgttgc gcccggattg tgtcaccagc aatacagaag tgttcttgct gttctccaac 3720
tttgacaacg gaaagagacc ctctacgcta caccagatga ataccaagct gagtgccgtg 3780
tatgccggag aagccatgca cacggccggg tgtgcaccat cctacagagt taagagagca 3840
gacatagcca cgtgcacaga agcggctgtg gttaacgcag ctaacgcccg tggaactgta 3900
gggatggccg tatgcagggc cgtggcgaag aaatggccgt cagccttaa gggagcagca 3960
acaccagtgg gcacaattaa aacagtcatg tgcggctcgt accccgtcat ccacgctgta 4020
gcgctaatt tctctgccac gactgaagcg gaaggggacc gcgaattggc cgctgttac 4080
cgggcagtgg ccgccgaagt aaacagactg tcactgagca gcgtagccat cccgctgctg 4140
tccacaggag tgttcagcgg cggaagagat aggctgcagc aatcctcaa ccatctattc 4200
acagcaatgg acgccacgga cgctgacgtg accatctact gcagagacaa aagttgggag 4260
aagaaatcc aggaagccat tgacatgagg acggctgtgg agttgctcaa tgatgacgtg 4320
gagctgacca cagacttggt gagagtgcac ccggacagca gcctggtggg tcgtaagggc 4380
tacagtacca ctgacgggtc gctgtactcg tactttgaag gtacgaaatt caaccaggct 4440
gctattgata tggcagagat actgacgttg tggcccagac tgcaagaggc aaacgaacag 4500
atatgcctat acgcgctggg cgaaacaatg gacaacatca gatccaaatg tccggtgaac 4560
gattccgatt catcaacacc tcccaggaca gtgccctgcc tgtgccgcta cgcaatgaca 4620
```

FIG.3D

```
gcagaacgga tcgcccgcct taggtcacac caagttaaaa gcatggtggt ttgctcatct 4680
ttcccctcc cgaaatacca tgtagatggg gtgcagaagg taaagtgcga gaaggttctc 4740
ctgttcgacc cgacggtacc ttcagtggtt agtccgcgga agtatgccgc atctacgacg 4800
gaccactcag atcggtcgtt acgagggttt gacttggact ggacccacga ctcgtcttcc 4860
actgccagcg ataccatgtc gctacccagt ttgcagtcgt gtgacatcga ctcgatctac 4920
gagccaatgg ctcccatagt agtgacggct gacgtacacc ctgaacccgc aggcatcgcg 4980
gacctggcgg cagatgtgca ccctgaaccc gcagaccatg tggacctcga gaacccgatt 5040
cctccaccgc gcccgaagag agctgcatac cttgcctccc gcgcggcgga gcgaccggtg 5100
ccggcgccga gaagccgac gcctgcccca aggactgcgt ttaggaacaa gctgcctttg 5160
acgttcggcg actttgacga gcacgaggtc gatgcgttgg cctccgggat tactttcgga 5220
gacttcgacg acgtcctgcg actaggccgc gcgggtgcat atatttctc ctcggacact 5280
ggcagcggac atttacaaca aaaatccgtt aggcagcaca atctccagtg cgcacaactg 5340
gatgcggtcc aggaggagaa aatgtacccg ccaaaattgg atactgagag ggagaagctg 5400
ttgctgctga aaatgcagat gcacccatcg gaggctaata agagtcgata ccagtctcgc 5460
aaagtggaga acatgaaagc cacggtggtg gacaggctca catcgggggc cagattgtac 5520
acgggagcgg acgtaggccg cataccaaca tacgcggttc ggtacccccg ccccgtgtac 5580
tcccctaccg tgatcgaaag atctccaagc cccgatgtag caatcgcagc gtgcaacgaa 5640
tacctatcca gaaattaccc aacagtggcg tcgtaccaga taacagatga atacgacgca 5700
tacttggaca tggttgacgg gtcggatagt tgcttggaca gagcgacatt ctgcccggcg 5760
aagctccggt gctacccgaa acatcatgcg taccaccagc cgactgtacg cagtgccgtc 5820
ccgtcaccct ttcagaacac actacagaac gtgctagcgg ccgccaccaa gatgaactgc 5880
aacgtcacgc aaatgcgaga actacccacc atggactcgg cagtgttcaa cgtggagtgc 5940
ttcaagcgct atgcctgctc cggagaatat tgggaagaat atgctaaaca acctatccgg 6000
ataaccactg agaacatcac tacctatgtg accaaattga aaggcccgaa agctgctgcc 6060
ttgttcgcta agacccacaa cttggttccg ctgcaggagg ttcccatgga cagattcacg 6120
gtcgacatga aacgagatgt caaagtcact ccagggacga aacacacaga ggaaagaccc 6180
```

FIG.3E

```
aaagtccagg taattcaagc agcggagcca ttggcgaccg cttacctgtg cggcatccac 6240
agggaattag taaggagact aaatgctgtg ttacgcccta acgtgcacac attgtttgat 6300
atgtcggccg aagactttga cgcgatcatc gcctctcact tccacccagg agacccggtt 6360
ctagagacgg acattgcatc attcgacaaa agccaggacg actccttggc tcttacaggt 6420
ttaatgatcc tcgaagatct agggctggat cagtacctgc tggacttgat cgaggcagcc 6480
ttgtgggaaa tatccagctg tcacctacca actggcacgc gcttcaagtt cggagctatg 6540
atgaaatcgg gcatgtttct gacttgttt attaacactg tttgaacat caccatagca 6600
agcagggtac tggagcagag actcactgac tccgcctgtg cggccttcat cggcgacgac 6660
aacatcgttc acggagtgat ctccgacaag ctgattggcgg agaggtgcgc gtcgtgggtc 6720
aacatggagg tgaagatcat tgacgctgtc atgggcgaaa aacccccata tttttgtggg 6780
ggattcatag ttttgacag cgtcacacag accgcctgcc gtgtttcaga cccacttaag 6840
cgcctgttca agttgggtaa gccgctaaca gctgaagaca agcaggacga agacaggcga 6900
cgagcactga gtgacgaggt tagcaagtgg ttccggacag gcttgggggc cgaactggag 6960
gtggcactaa catctaggta tgaggtagag ggctgcaaaa gtatcctcat agccatggcc 7020
acctggcga gggacattaa ggcgtttaag aaattgagag gacctgttat acacctctac 7080
ggcggtccta gattggtgcg ttaatacaca gaattctgat tggatcatag cgcatatta 7140
taggatccag atcccgggta attaatgaa ttacatcccc acgcaaacgt ttacggccg 7200
ccggtggcgc ccgcgcccgg cggcccgtcc ttggccgttg caggccactc cggtggctcc 7260
cgtcgtcccc gacttccagg cccagcagat gcagcaactc atcagcgccg taaatgcgct 7320
gacaatgaga cagaacgcaa ttgctcctgc taggcctccc aaaccaaaga agaagaagac 7380
aaccaaacca aagccgaaaa cgcagcccaa gaagatcaac ggaaaaacgc agcagcaaaa 7440
gaagaaagac aagcaagccg acaagaagaa gaagaaaccc ggaaaaagag aaagatgtg 7500
catgaagatt gaaaatgact gtatcttcgt atgcggctag ccacagtaac gtagtgttc 7560
cagacatgtc gggcaccgca ctatcatggg tgcagaaaat ctgggtggt ctgggggcct 7620
tcgcaatcgg cgctatcctg gtgctggttg tggtcacttg catgggctc cgcagataag 7680
ttagggtagg caatggcatt gatatagcaa gaaaattgaa aacagaaaaa gttagggtaa 7740
```

FIG.3F

```
gcaatggcat ataaccataa ctgtataact tgtaacaaag cgcaacaaga cctgcgcaat 7800
tggccccgtg gtccgcctca cggaaactcg gggcaactca tattgacaca ttaattggca 7860
ataattggaa gcttacataa gcttaattcg acgaataatt ggatttttat tttattttgc 7920
aattggtttt taatatttcc aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 7980
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa                                  8010
```

FIG.4A

Nucleotide sequence of pSFVlink

```
gatggcggat gtgtgacata cacgacgcca aaagattttg ttccagctcc tgccacctcc 60
gctacgcgag agattaacca cccacgatgg ccgccaaagt gcatgttgat attgaggctg 120
acagcccatt catcaagtct ttgcagaagg cattccgtc gttcgaggtg gagtcattgc 180
aggtcacacc aaatgaccat gcaaatgcca gagcatttc gcacctggct accaaattga 240
tcgagcagga gactgacaaa gacacactca tcttggatat cggcagtgcg cctccagga 300
gaatgatgtc tacgcacaaa taccactgcg tatgccctat gcgcagcgca gaagacccg 360
aaaggctcga tagctacgca aagaaactgg cagcggcctc cgggaaggtg ctggatagag 420
agatcgcagg aaaaatcacc gacctgcaga ccgtcatggc tacgccagac gctgaatctc 480
ctacttttg cctgcatata gacgtcacgt gtcgtacggc agccgaagtg atctacattg 540
aggacgtgta tgctgtacat gcaccaacat cgctgtacca tcaggcgatg aaaggtgtca 600
gaacggcgta ttggattggg tttgacacca ccccgtttat gtttgacgcg ctagcaggcg 660
cgtatccaac ctacgccaca aactgggccg acgagcaggt gttacagggcc aggaacatag 720
gactgtgtgc agcatccttg actgagggaa gactcggcaa actgtccatt ctccgcaaga 780
agcattgaa acctgcgac acagtcatgt tctcggtagg atctacattg tacactgaga 840
gcagaaagct actgaggagc tggcacttac cctccgtatt ccacctgaaa ggtaaacaat 900
cctttacctg taggtgcgat accatcgtat catgtgaagg gtacgtagtt aagaaaatca 960
ctatgtgccc cggcctgtac ggtaaaacgg tagggtacgc cgtgacgtat cacgcgggagg 1020
gattcctagt gtgcaagacc acagacactg tcaaaggaga aagagtctca ttccctgtat 1080
gcacctacgt cccctcaacc atctgtgatc aaatgactgg catactagcg accgacgtca 1140
caccggagga cgcacagaag ttgttagtgg gattgaatca gaggatagtt gtgaacggaa 1200
gaacacagcg aaacactaac acgatgaaga actatctgct tccgattgtg gccgtcgcat 1260
ttagcaagtg ggcgagggaa tacaaggcag acttgatga tgaaaaacct ctggtgtccc 1320
gagaggttc acttacttgc tgctgcttgt gggcatttaa aacgaggaag atgcacacca 1380
tgtacaagaa accagacacc cagacaatag tgaaggtgcc ttcagagttt aactcgttcg 1440
```

FIG.4B

```
tcatcccgag cctatggtct acaggcctcg caatcccagt cagatcacgc attaagatgc 1500
ttttggccaa gaagaccaag cgagagttaa tacctgtttct cgacgcgtcg tcagccaggg 1560
atgctgaaca agaggagaag gagaggttgg aggccgagct gactagagaa gccttaccac 1620
ccctcgtccc catcgcgccg gcggagacgg gagtcgtcga cgtcgacgtt gaagaactag 1680
agtatcacgc aggtgcaggg gtcgtggaaa cacctcgcag cgcgttgaaa gtcaccgcac 1740
agccgaacga cgtactacta ggaaattacg tagttctgtc cccgcagacc gtgctcaaga 1800
gctccaagtt ggccccctg caccctctag cagagcaggt gaaaataata acacataacg 1860
ccgacgagga gaactacgag aaagtcagag ctgaaagaac tgacgccgag tacgtgttcg 2100
acgtagataa aaaatgctgc gtcaagagag aggaagcgtc gggtttggtg ttggtgggag 2160
agctaaccaa ccccccgttc catgaattcg cctacgaagg gctgaagatc aggccgtcgg 2220
caccatataa gactacagta gtaggagtct ttgggggttcc gggatcaggc aagtctgcta 2280
ttattaagag cctcgtgacc aaacacgatc tggtcaccag cggcaagaag gagaactgcc 2340
aggaaatagt taacgacgtg aagaagcacc gcgggaaggg gacaagtagg gacgaggctt 2460
actccatcct gctaaacggg tgtcgtcgtg ccgtggacat cctatatgtg gacgaggctt 2460
tcgcttgcca ttccggtact ctgctggccc taattgctct tgttaaacct cggagcaaag 2520
tggtgttatg cggagacccc aagcaatgcg gattcttcaa tatgatgcag cttaaggtga 2580
acttcaacca caacatctgc actgaagtat gtcataaaag tatatccaga cgttgcacgc 2640
gtccagtcac ggccatcgtg tctacgttgc actacggagg caagatgcgc acgaccaacc 2700
cgtgcaacaa acccataatc atagacacca caggacagac caagcccaag ccaggagaca 2760
tcgtgttaac atgcttccga ggctgggcaa agcagctgca gttggactac cgtggacacg 2820
aagtcatgac agcagcagca tctcagggcc tcacccgcaa agggggtatac gccgtaaggc 2880
agaaggtgaa tgaaaatccc ttgtatgccc ctgcgtcgga gcacgtgaat gtactgctga 2940
cgcgcactga ggataggctg gtgtggaaaa cgctggccgg cgatccctgg attaaggtcc 3000
```

FIG.4C

```
tatcaaacat tccacagggt aactttacgg ccacattgga agaatggcaa gaagaacacg 3060
acaaaataat gaaggtgatt gaaggaccgg ctgcgcctgt ggacgcgttc cagaacaaag 3120
cgaacgtgtg ttgggcgaaa agcctggtgc ctgtcctgga cactgccgga atcagattga 3180
cagcagagga gtggagcacc ataattacag catttaagga ggacagagct tactctcag 3240
tggtggcctt gaatgaaatt tgcaccaagt actatggagt tgacctggac agtggcctgt 3300
tttctgcccc gaaggtgtcc ctgtattacg agaacaacca ctgggataac agacctggtg 3360
gaaggatgta tggattcaat gccgcaacag ctgccaggct ggaagctaga catacctccc 3420
acgtcctgct ggtgagtgag tacaacctgg ctttgccctg acgcagggtc acttggttgt 3660
caccgctgaa tgtcacaggc gccgataggt gctacgacct aagtttagga ctgcggctg 3720
acgccggcag gttcgacttg gtctttgtga acattcacac ggaattcaga atccaccact 3780
accagcagtg tgtcgaccac gccatgaagc tgcagatgct tgggggagat gcgctacgac 3840
tgctaaaacc cggcggcatc ttgatgagag cttacggata cgccgataaa atcagcgaag 3900
ccgttgtttc ctccttaagc agaaagttct cgtctgcaag agtgttgcgc ccggattgtg 3960
tcaccagcaa tacagaagtg ttcttgctgt tctccaactt tgacaacgga aagagaccct 4020
ctacgctaca ccagatgaat accaagctga gtgccgtgta tgccggagaa gccatgcaca 4080
cggccgggtg tgcaccatcc tacagagtta agagagcaga catagccacg tgcacagaag 4140
cggctgtggt taacgcagct aacgcccgtg gaactgtagg ggatggcgta tgcagggccg 4200
tggcgaagaa atggccgtca gcctttaagg gagcagcaac accagtgggc acaattaaaa 4260
cagtcatgtg cggctcgtac cccgtcatcc acgctgtagc gcctaatttc tctgccacga 4320
ctgaagcgga aggggaccgc gaattggccg ctgctaccgg ggcagtggcc gccgaagtaa 4380
acagactgtc actgagcagc gtagccatcc cgctgctgtc cacaggagtg ttcagcggcg 4440
gaagagatag gctgcagcaa tccctcaacc atctattcac agcaatggac gccacggacg 4500
ctgacgtgac catctactgc agagacaaaa gttgggagaa gaaaatccag gaagccattg 4560
```

FIG.4D

```
acatgaggac ggctgtggag ttgctcaatg atgacgtgga gctgaccaca gacttggtga 4620
gagtgcaccc ggacagcagc ctggtgggtc gtaagggcta cagtaccact gacgggtcgc 4680
tgtactccgta ctttgaaggt acgaaattca accaggctgc tattgatatg gcagagatac 4740
tgacgttgtg gcccagactg caagaggcaa acgaacagat atgcctatac gcgctgggcg 4800
aaacaatgga caacatcaga tccaaatgtc cggtgaacga ttccgattca tcaacacctc 4860
ccaggacagt gccctgcctg tgccgctacg caatgacagc agaacggatc gcccgcctta 4920
ggtcacacca agttaaaagc atggtggttt gctcatcttt tcccctcccg aaataccatg 4980
tagatgggct gcagaaggta aagtgcgaga aggttctcct gttcgacccg acggtacctt 5040
cagtggttag tccgcggaag tatgccgcat ctacgacgga ccactcagat cggtcgttac 5100
gagggtttga cttggactgg accaccgact cgtcttccac tgccagcgat accatgtcgc 5160
tacccagttt gcagtcgtgt gacatcgact cgatctacga gccaatggct cccatagtag 5220
tgacggctga cgtacaccct gaacccgcag gcatcgcgga cctggcggca gatgtgcacc 5280
ctgaacccgc agaccatgtg gacctcgaga acccgattcc tccaccgcgc ccgaagagag 5340
ctgcatacct tgcctcccgc gcggcggagc gaccgtgccc ggcgccgaga aagccgacgc 5400
ctgccccaag gactgcgttt aggaacaagc tgcctttgac gttcggcgac ttgacgagc 5460
acgaggtcga tgcgttggcc tccgggatta ctttcggaga cttcgacgac gtcctgcgac 5520
taggccgcgc gggtgcatat attttctcct cggacactgg cagcggacat ttacaacaaa 5580
aatcgttag gcagcacaat ctccagtgcg cacaactgga tgcggtccag gaggagaaaa 5640
tgtacccgcc aaaattggat actgagaggg agaagctgtt gctgctgaaa atgcagatgc 5700
acccatcgga ggctaataag agtcgatacc agtctcgcaa agtggagaac atgaaagcca 5760
cggtggtgga caggctcaca tcgggggcca gattgtacac gggagcggac gtaggccgca 5820
taccaacata cgcggttcgg taccccccgc ccgtgtactc ccctaccgtg atcgaaagat 5880
tctcaagccc cgatgtagca atcgcagcgt gcaacgaata cctatccaga aattacccaa 5940
cagtggcgtc gtaccagata acagatgaat acgacgcata cttggacatg gttgacgggt 6000
cggatagttg cttggacaga gcgacattct gcccggcgaa gctccggtgc taccgaaac 6060
atcatgcgta ccaccagccg actgtacgca gtgccgtccc gtcaccctt cagaacacac 6120
```

FIG.4E

```
tacagaacgt gctagcggcc gccaccaaga gaaactgcaa cgtcacgcaa atgcgagaac 6180
taccaccaat ggactcggca gtgttcaacg tggagtgctt caagcgctat gcctgctccg 6240
gagatattg ggaagaatat gctaaacaac ctatccggat aaccactgag aacatcacta 6300
cctatgtgac caaattgaaa ggcccgaaag ctgctgcctt gttcgctaag acccacaact 6360
tggttccgct gcaggaggtt cccatggaca gattcacgat cgacatgaaa cgagatgtca 6420
aagtcactcc agggacgaaa cacacagagg aaagacccaa agtccaggta attcaagcag 6480
cggagccatt ggcgaccgct tacctgtgcg gcatccacag ggaattagta aggagactaa 6540
atgctgtgtt acgccctaac gtgcacacat tgtttgatat gtcggccgaa gactttgacg 6600
cgatcatcgc ctctcacttc cacccaggag acccggttct agagacggac attgcatcat 6660
tcgacaaaag ccaggacgac tccttggctc ttacaggttt aatgatcctc gaagatctag 6720
gggtggatca gtacctgctg gacttgatcg aggcagcctt tggggaaata tccagctgtc 6780
acctaccaac tggcacgcgc ttcaagttcg gagctatgat gaaatcgggc atgtttctga 6840
ctttgtttat taacactgtt ttgaacatca ccatagcaag cagggtactg gagcagagac 6900
tcactgactc cgcctgtgcg gccttcatcg gcgacgacaa catcgttcac ggagtgatct 6960
ccgacaagct gatggcggag aggtgcgcgt cgtgggtcaa catggaggtg aagatcattg 7020
acgctgtcat gggcgaaaaa ccccccatatt tttgtgtggg attcatagtt tttgacagcg 7080
tcacacagac cgcctgccgt gtttcagacc cacttaagcg cctgttcaag ttgggtaagc 7140
cgctaacagc tgaagacaag caggacgaag acaggcgacg agcactgagt gacgaggtta 7200
gcaagtggtt ccggacaggc ttgggggccg aactggaggt ggcactaaca tctaggtatg 7260
aggtagaggg ctgcaaaagt atcctcatag ccatggccac cttggcgagg gacattaagg 7320
cgttaagaa attgagagga cctgttatac acctctacgg cggtcctaga ttggtgcgtt 7380
aatacacaga attctgattg gatcatagcg cactattata ggatccagat cccgggtaat 7440
taattgaatt acatcccctac gcaaacgttt tacggccgcc ggtggcgccc gcgcccggcg 7500
gcccgtcctt ggccgttgca ggccactccg gtggctcccg tcgtccccga cttccaggcc 7560
cagcgatgc agcaactcat cagcgccgta aatgcgctga caatgagaca gaacgcaatt 7620
gctcctgcta ggcctcccaa accaaagaag aagaagacaa ccaaaccaaa gccgaaaacg 7680
```

FIG.4F

```
cagcccaaga agatcaacgg aaaaacgcag cagcaaaaga agaaagacaa gcaagccgac 7740
aagaagaaga agaaacccgg aaaaagagaa agaatgtgca tgaagattga aaatgactgt 7800
atcttcgtat gcggctagcc acagtaacgt agtgtttcca gacatgtcgg gcaccgcact 7860
atcatgggtg cagaaatct cgggtggtct ggggcccttc gcaatcggcg ctatcctggt 7920
gctggttgtg gtcacttgca ttgggctccg cagataagtt agggtaggca atggcattga 7980
tatagcaaga aaattgaaaa cagaaaaagt tagggtaagc aatggcatat aaccataact 8040
gtataacttg taacaaagcg caacaagacc tgcgcaattg gcccgtggt ccgcctcacg 8100
gaaactcggg gcaactcata ttgacacatt aattggcaat aattggaagc ttacataagc 8160
ttaattcgac gaataattgg attttattt tatttgcaa ttggttttta atatttccaa 8220
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 8280
aaaaaaaaa aaaaaaaaaa ccaacgcgcg gggagaggcg gtttgcgtat 8340
aaaaaaact agtctgcatt aatgaatcgg ctcgctgcgc tcggtcgttc ggctgcggcg 8400
tgggcgtct tccgcttcct cgctcactga ctcgctgcgc tcggtcgttc ggctgcggcg 8460
agcggtatca gctcactcaa aggcggtaat acggttatcc acagaatcag gggataacgc 8520
aggaaagaac atgtgagcaa aaggccagca aaaggccagg aaccgtaaaa aggccgcgtt 8580
gctggcgttt ttccataggc tccgcccccc tgacgagcat cacaaaaatc gacgctcaag 8640
tcagaggtgg cgaaacccga caggactata aagataccag gcgtttcccc ctggaagctc 8700
cctcgtgcgc tctcctgttc cgaccctgcc gcttaccgga tacctgtccg cctttctccc 8760
ttcgggaagc gtggcgcttt ctcaatgctc gcgctgtagg tatctcagtt cggtgtaggt 8820
cgttcgctcc aagctgggct gtgtgcacga accccccgtt cagcccgacc gctgcgcctt 8880
atccggtaac tatcgtcttg agtccaaccc ggtaagacac gacttatcgc cactggcagc 8940
agccactggt aacaggatta gcagagcgag gtatgtaggc ggtgctacag agttcttgaa 9000
gtggtggcct aactacggct acactagaag gacagtattt ggtatctgcg ctctgctgaa 9060
gccagttacc ttcggaaaaa gagttggtag ctcttgatcc ggcaaacaaa ccaccgctgg 9120
tagcggtggt tttttgtttt gcaagcagca gattacgcgc agaaaaaaag gatctcaaga 9180
agatccttcg atcttttcta cggggtctga cgctcagtgg aacgaaaact cacgttaagg 9240
gatttggtc atgagattat caaaaaggat cttcacctag atcctttaa attaaaatg 9240
```

FIG.4G

```
aagttttaaa tcaatctaaa gtatatatga gtaaacttgg tctgacagtt accaatgctt 9300
aatcagtgag gcacctatct cagcgatctg tctatttcgt tcatccatag ttgcctgact 9360
ccccgtcgtg tagataacta cgatacggga gggcttacca tctggcccca gtgctgcaat 9420
gataccgcga gacccacgct caccggctcc agatttatca gcaataaacc agccagccgg 9480
aagggccgag cgcagaagtg gtcctgcaac tttatccgcc tccatcccagt ctattaattg 9540
ttgccgggaa gctagagtaa gtagttcgcc agtttaatagt ttgcgcaacg ttgttgccat 9600
tgctacaggc atcgtggtgt cacgctcgtc gtttggtatg gcttcattca gctccggttc 9660
ccaacgatca aggcgagtta catgatcccc catgttgtgc aaaaaagcgg ttagctcctt 9720
cggtcctccg atcgttgtca gaagtaagtt ggccgcagtg ttatcactca tggttatggc 9780
agcattgcat aattctctta ctgtcatgcc atccgtaaga tgcttttctg tgactggtga 9840
gtactcaacc aagtcattct gagaatagtg tatgcggcga ccgagttgct cttgcccggc 9900
gtcaatacgg gataaaccg cgccacatag cagaacttta aaagtgctca tcattggaaa 9960
acgttcttcg gggcgaaaac tctcaaggat cttaccgctg ttgagatcca gttcgatgta 10020
acccactcgt gcacccaact gatcttcagc atcttttact ttcaccagcg tttctgggtg 10080
agcaaaaaca ggaaggcaaa atgccgcaaa aaagggaata agggcgacac ggaaatgttg 10140
aatactcata ctcttcttt ttcaatatta ttgaagcatt tatcagggtt atgtctcat 10200
gagcggatac atatttgaat gtatttagaa aaataaacaa atagggttc cgcgcacatt 10260
tcccgaaaa gtgccacctg acgtctaaga aaccattatt atcatgacat taacctataa 10320
aaataggcgt atcacgaggc cctttcgtct cgcgcgtttc ggtgatgacg gtgaaaacct 10380
ctgacacatg cagctcccgg agacggtcac agcttctgtc taagcggatg ccgggagcag 10440
acaagcccgt cagggcgcgt tgagcgggtgt tggcgggtgt cggggctggc ttaactatgc 10500
ggcatcagag cagattgtac tgagagtgca ccatatcgac gctctcccct atgcgactcc 10560
tgcattagga agcagcccag tactaggttg aggccgttga gcaccgccgc cgcaaggaat 10620
ggtgcatgca aggagatggc gcccaacagt cccccggcca cggggcctgc caccataccc 10680
acgccgaaac aagcgctcat gagcccgaag tggcgagccc gatcttcccc atcggtgatg 10740
tcggcgatat aggcgccagc aaccgcacct gtggcgccgg tgatgccggc cacgatgcgt 10800
```

FIG.4H

```
ccggcgtaga ggatctggct agcgatgacc ctgctgattg gttcgctgac catttcccgg 10860
gtgcggaacg gcgttaccag aaactcagaa ggttcgtcca accaaaccga ctctgacggc 10920
agtttacgag agagatgata gggtctgctt cagtaagcca gatgctacac aattaggctt 10980
gtacatattg tcgttagaac gcggctacaa ttaatacata accttatgta tcatacacat 11040
acgatttagg tgacactata                                           11060
```

17/39

Construction of pSFVlink

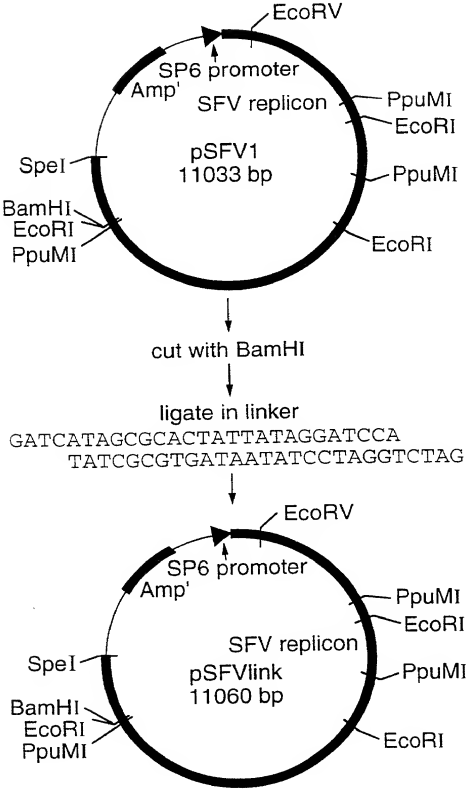

FIG.5

FIG.6A

Nucleotide Sequence of pMP76

```
attggctatt ggccattgca tacgttgtat ctatatcata atatgtacat ttatattggc 60
tcatgtccaa tatgaccgcc atgttgacat tgattattga ctagttatta atagtaatca 120
attacggggt cattagttca tagcccatat atggagttcc gcgttacata acttacggta 180
aatggcccgc ctcgtgaccg cccaacgacc cccgcccatt gacgtcaata atgacgtatg 240
ttcccatagt aacgccaata gggactttcc attgacgtca atgggtggag tatttacggt 300
aaactgccca cttggcagta catcaagtgt atcatatgcc aagtccgccc cctattgacg 360
tcaatgacgg taaatggccc gcctggcatt atgcccagta catgaccttc cgggactttc 420
ctacttggca gtacatctac gtattagtca tcgctattac catggtgatg cggttttggc 480
agtacaccaa tgggcgtgga tagcggtttg actcacgggg atttccaagt ctccacccca 540
ttgacgtcaa tgggagtttg tttggcacc aaaatcaacg ggactttcca aaatgtcgta 600
ataacccccgc cccgttgacg caaatgggcg gtaggcgtgt acggtgggag gtctatataa 660
gcagagctcg tttagtgaac cgtatgggcg atgtgtgaca tacacgacgc caaaagattt 720
tgttccagct cctgccacct ccgctacgcg agagattaac cacccacgat ggccgccaaa 780
gtgcatgttg atattgaggc tgacagccca ttcatcaagt ctttgcagaa ggcatttccg 840
tcgttcgagg tggagtcatt gcaggtcaca ccaaatgacc atgcaaatgc cagagcattt 900
tcgcacctgg ctaccaaatt gatcgagcag gagactgaca aagacacact catttggat 960
atcggcagtg cgccttccag gagaatgatg tctacgcaca aataccactg cgtatgccct 1020
atgcgcagcg cagaagaccc cgaaaggttc gatagctacg caaagaaact ggcagcggcc 1080
tccgggaagg tgctggatag agagatcgca ggaaaaatca ccgacctgca gaccgtcatg 1140
gctacgccag acgctgaatc tcctaccttt tgcctgcata cagacgtcac gtgtcgtacg 1200
gcagccgaag tggccgtata ccaggacgtg tatgctgtac atgcaccaac atcgctgtac 1260
catcaggcga tgaaaggtgt cagaacggcg tattggattg ggtttgacac cacccgttt 1320
atgtttgacg cgctagcagg cgcgtatcca acctacgcca caaactgggc cgacgagcag 1380
```

FIG.6B

```
gtgttacagg ccaggaacat aggactgtgt gcagcatcct tgactgaggg aagactcggc 1440
aaactgtcca ttctccgcaa gaagcaattg aaaccttgcg acacagtcat gttctcggta 1500
ggatctacat tgtacactga gagcagaaag ctactgagga gctggcactt accctccgta 1560
ttccacctga aaggtaaaca atcctttacc tgtaggtgcg ataccatcgt atcatgtgaa 1620
gggtacgtag ttaagaaaat cactatgtgc cccggcctgt acggtaaaac ggtagggtac 1680
gccgtgacgt atcacgcgga gggattccta gtgtgcaaga ccacagacac tgtcaaagga 1740
gaaagagtct cattcctgt atgcacctac gtcccctcaa ccatctgtga tcaaatgact 1800
ggcatactag cgaccgacgt cacaccggag gacgcacaga agttgttagt gggattgaat 1860
cagaggatag ttgtgaacgg aagaacacag cgaaacacta acacgatgaa gaactatctg 1920
cttccgattg tggccgtcgc atttagcaag tgggcgaggg aatacaaggc agaccttgat 1980
gatgaaaaac ctctgggtgt ccgagagagg tcacttactt gctgctgctt gtgggcattt 2040
aaaacgagga agatgcacac catgtacaag aaaccagaca cccagacaat agtgaaggtg 2100
ccttcagagt ttaactcgtt cgtcatcccg agcctatggt ctacaggcct cgcaatccca 2160
gtcagatcac gcattaagat gctttggcc aagaagacca agcgagagtt gaaccttgtt 2220
ctcgacgcgt cgtcagccag ggatgctgaa caagaggaga aggagaggtt ggaggccgag 2280
ctgactagag aagccttacc accctcgtc cccatcgcgc cggcggagac gggagtcgtc 2340
gacgtcgacg ttgaagaact agagtatcac gcaggtgcag gggtcgtgga aacacctcgc 2400
agcgcgttga aagtcaccgc acagccgaac gacgtactac tagaaatta cgtagtttg 2460
tccccgcaga ccgtgctcaa gagctccaag ttggcccccg tgcaccctct agcagagcag 2520
gtgaaaataa taacacataa cgggagggcc ggcggttacc aggtcgacgg atatgacggc 2580
agggtcctac taccatgtgg atcggccatt ccggtccctg agtttcaagc tttgagcgag 2640
agcgccacta tggtgtacaa cgaaagggag ttcgtcaaca ggaaactata ccatattgcc 2700
gttcacggac cgtcgctgaa caccgacgag gagaactacg agaaagtcag agctgaaaga 2760
actgacgccg agtacgtgtt cgacgtagat aaaaaatgct gcgtcaagag agaggaagcg 2820
tcgggtttgg tgttggtggg agagctaacc aacccccgt tccatgaatt cgcctacgaa 2880
gggctgaaga tcaggccgtc ggcaccatat aagactacag tagtaggagt ctttgggggtt 2940
```

FIG.6C

ccgggatcag	gcaagtctgc	tattattaag	agcctcgtga	ccaaacacga	tctggtcacc	3000
agcggcaaga	aggagaactg	ccaggaaata	gttaacgacg	tgaagaagca	ccgcgggaag	3060
ggacaagta	gggaaaacag	tgactccatc	ctgctaaacg	ggtgtcgtcg	tgccgtggac	3120
atcctatatg	tggacgaggc	tttcgcttgc	cattccggta	ctctgctagc	cctaattgct	3180
cttgttaaac	ctcggagcaa	agtggtgtta	tgcggagacc	ccaagcaatg	cggattcttc	3240
aatatgatgc	agcttaaggt	gaacttcaac	cacaacatct	gcactgaagt	atgtcataaa	3300
agtatatcca	gacgttgcac	gcgtccagtc	acggccatcg	tgtctacgtt	gcactacgga	3360
ggcaagatgc	gcacgaccaa	cccgtgcaac	aaacccataa	tcatagacac	cacaggacag	3420
accaagccca	agccaggaga	catcgtgtta	acatgcttcc	gaggctgggc	aaagcagctg	3480
cagttggact	accgtggaca	cgaagtcatg	acagcagcag	catctcaggg	cctcacccgc	3540
aaaggggtat	acgccgtaag	gcagaaggtg	aatgaaaatc	cttgtatgtc	ccctgcgtcg	3600
gagcacgtga	atgtactgct	gacgcgcact	gaggataggc	tggtgtggaa	aacgctggcc	3660
ggcgatccct	ggattaaggt	gagtttgggg	accctgatt	gttctttctt	tttcgctatt	3720
gtaaaattca	tgttatatgg	aggggcaaa	gttttcaggg	tgttgtttag	aatgggaaga	3780
tgtccttgt	atcaccatgg	accctcatga	taattttgtt	tctttcactt	tctactctgt	3840
tgacaaccat	tgtctcctct	tatttttctt	tcatttttctg	taacttttttc	gttaaacttt	3900
agcttgcatt	tgtaacgaat	ttttaaattc	actttgttt	atttgtcaga	ttgtaagtac	3960
ttctctaat	cacttttttt	tcaaggcaat	cagggtatat	tatattgtac	ttcagcacag	4020
tttagagaa	caattgttat	aattaaatga	taaggtagaa	tatttctgca	tataaattct	4080
ggctggcgtg	gaaatattct	tattggtaga	aacaactaca	tcctggtcat	catcctgcct	4140
ttctctttat	ggttacaatg	atatacactg	tttgagatga	ggataaaata	ctctgagtcc	4200
aaaccggccc	cctctgctaa	ccatgttcat	gccttcttct	ttttcctaca	ggtcctatca	4260
aacattccac	agggtaactt	tacggccaca	ttggaagaat	ggcaagaaga	acacgacaaa	4320
ataatgaagg	tgattgaagg	accggctgcg	cctgtggacg	cgttccagaa	caaagcgaac	4380
gtgtgttggg	cgaaaagcct	ggtgcctgtc	ctggacactg	ccggatcag	attgacagca	4440
gaggagtgga	gcaccataat	tacagcattt	aaggaggaca	gagcttactc	tccagtggtg	4500

FIG.6D

```
gccttgaatg aaatttgcac caagtactat ggagttgacc tggacagtgg cctgttttct 4560
gccccgaagg tgtccctgta ttacgagaac aaccactggg ataacagacc tggtggaagg 4620
atgtatggat tcaatgccgc aacagctgcc aggctggaag ctagacatac cttcctgaag 4680
gggcagtggc atacgggcaa gcaggcagtt atcgcagaaa gaaaaatcca accgctttct 4740
gtgctggaca atgtaattcc tatcaaccgc aggctgccgc acgccctggt ggctgagtac 4800
aagacggtta aaggcagtag ggttgagtgg ctggtcaata aagtaagagg gtaccacgtc 4860
ctgctggtga gtgagtacaa cctggctttg cctcgacgca gggtcacttg gttgtcaccg 4920
ctgaatgtca caggcgccga taggtgctac gacctaagtt taggactgcc ggctgacgcc 4980
ggcaggttcg acttggtctt tgtgaacatt cacacggaat tcagaatcca ccactaccag 5040
cagtgtgtcg accacgccat gaagctgcag atgcttgggg gagatgcgct acgactgcta 5100
aaacccggcg gcatcttgat gagagcttac ggatacgccg ataaaatcag cgaagccgtt 5160
gtttcctcct taagcagaaa gttctcgtct gcaagagtgt tgcgcccgga ttgtgtcacc 5220
agcaatacag aagtgttctt gctgtttctcc aacttttgaca acggaaagag accctctacg 5280
ctacaccaga tgaataccaa gctgagtgcc gtgtatgccg gagaagccat gcacacggcc 5340
gggtgtgcac catcctacag agttaagaga gcagacatag ccacgtgcac agaagcggct 5400
gtggttaacg cagctaacgc ccgtggaact gtaggggatg gcgtatgcag ggccgtggcg 5460
aagaaatggc cgtcagcctt taagggagca gcaacaccag tgggcacaat taaaacagtc 5520
atgtgcggct cgtacccgt catccacgct gtagcgccta atttctctgc cacgactgaa 5580
gcggaagggg accgcgaatt ggccgctgtc taccgggcag tggccgccga agtaaacaga 5640
ctgtcactga gcagcgtagc catcccgctg ctgtccacag gagtgttcag cggcggaaga 5700
gataggctgc agcaatccct caaccatcta ttcacagcaa tggacgccac ggacgctgac 5760
gtgaccatct actgcagaga caaaagttgg gagaagaaaa tccaggaagc cattgacatg 5820
aggacggctg tggagttgct caatgatgac gtggagctga ccacagactt ggtgagagtg 5880
cacccggaca gcagcctggt gggtcgtaag ggctacagta ccactgacgg gtcgctgtac 5940
tcgtactttg aaggtacgaa attcaaccag gctgctattg atatggcaga gatactgacg 6000
ttgtggccca gactgcaaga ggcaaacgaa cagatatgcc tatacgcgct gggcgaaaca 6060
```

FIG.6E

```
atggacaaca tcagatccaa atgtccggtg aacgatccgg attcatcaac acctcccagg 6120
acagtgccct gcctgtgccg ctacgcaatg acagcagaac ggatcgcccg ccttaggtca 6180
caccaagtta aaagcatggt ggtttgctca tctttcccc tcccgaaata ccatgtagat 6240
ggggtgcaga aggtaaagtg cgagaaggtt ctccgttccg acccgacggt accttcagtg 6300
gttagtccgc ggaagtatgc cgcatctacg acggaccact cagatcggtc gttacgaggg 6360
ttgactttgg actggaccac cgactcgtct tccactgcca gcgataccat gtcgctaccc 6420
agtttgcagt cgtgtgacat cgactcgatc tacgagccaa tggctcccat agtagtgacg 6480
gctgacgtac accctgaacc cgcaggcatc gcggacctgg cggcagatgt gcaccctgaa 6540
cccgcagacc atgtggacct cgagaacccg attcctccac cgcgcccgaa gagagctgca 6600
taccttgcct cccgcgcggc ggagcgaccg gtgccgggcgc cgagaaagcc gacgcctgcc 6660
ccaaggactg cgtttaggaa caagctgcct ttgacgttcg gcgacttgga cgagcacgag 6720
gtcgatgcgt tggcctccgg gattactttc ggagacttcg acgacgtcct gcgactaggc 6780
acatacgcgg ttcggtaccc ccgccccgtg tactccccta ccgtgatcga aagatttcta 6840
agccccgatg tagcaatcgc agcgtgcaac gaatacctat ccagaaatta cccaacagtg 7200
gcgtcgtacc agataaacaga tgaatacgac gcatacttgg acatggttga cgggtcggat 7260
agttgcttgg acagagcgac attctgcccg gcgaagctcc ggtgctaccc gaaacatcat 7320
gcgtaccacc agccgactgt acgcagtgcc gtcccgtcac cctttcagaa cacactacag 7380
aacgtgctag cggccgccac caagagaaac tgcaacgtca cgcaaatgcg agaactaccc 7440
accatggact cggcagtgtt caacgtggag tgcttcaagc gctatgcctg ctccggagaa 7500
tattgggaag aatatgctaa acaacctatc cggataacca ctgagaacat cactacctat 7560
gtgaccaaat tgaaaggccc gaaagctgct gccttgttcg ctaagaccca caacttggtt 7620
```

FIG.6F

```
ccgctgcagg aggttcccat ggacagattc acggtcgaca tgaaacgaga tgtcaaagtc 7680
actccaggga cgaaacacac agaggaaaga cccaaagtcc aggtaattca agcagcggag 7740
ccattggcga ccgcttacct gtgcggcatc cacagggaat tagtaaggag actaaatgct 7800
gtgttacgcc ctaacgtgca cacattgttt gatatgtcgg ccgaagactt tgacgcgatc 7860
atcgcctctc acttccaccc aggagacccg gttctagaga cggacattgc atcattcgac 7920
aaaagccagg acgactcctt ggctcttaca ggttaatga tcctcgaaga tctaggggtg 7980
gatcagtacc tgctggactt gatcgaggca gccttgtggg aaatatccag ctgtcaccta 8040
ccaactggca cgcgcttcaa gttcggagct atgatgaaat cgggcatgtt tctgacttta 8100
tttattaaca ctgttttgaa catcaccata gcaagcaggg tactggagca gagactcact 8160
gactccgcct gtgcggcctt catcggcgac gacaacatcg ttcacggagt gatctccgac 8220
aagctgatgg cggagaggtg cgcgtcgtgg gtcaacatgg aggtgaagat cattgacgct 8280
gtcatgggcg aaaaacccc atatttttgt gggggattca tagttttga cagcgtcaca 8340
cagaccgcct gccgtgtttc agacccactt aagcgcctgt tcaagttggg taagccgcta 8400
acagctgaag acaagcagga cgagagcagg cgacgagcac tgagtgacga ggttagcaag 8460
tggttccgga caggcttggg ggccgaactg gaggtggcac taacatctag gtatgaggta 8520
gagggctgca aaagtatcct catagccatg gccaccttgg cgagggacat taaggcgttt 8580
aagaaattga gaggacctgt tatacacctc tacggcggtc ctagattggt gcgttaatac 8640
acagaattct gatggatca tagcgcacta ttataggatc cagatcccgg gtaattaatt 8700
gaattacatc cctacgcaaa cgtttacgg ccgccggtgg cgcccgcgcc cggcggcccg 8760
tccttggccg ttgcaggcca ctccggtggc tcccgtcgtc cccgacttcc aggcccagca 8820
gatgcagcaa ctcatcagcg ccgtaaatgc gctgacaatg agacagaacg caattgctcc 8880
tgctaggcct cccaaaccaa agaagaagaa gacaaccaaa ccaaagccga aaacgcagcc 8940
caagaagatc aacggaaaaa cgcagcagca aaagaagaaa gacaagcaag ccgacaagaa 9000
gaagaagaaa cccggaaaaa gagaaagaat gtgcatgaag attgaaaatg actgtatctt 9060
cgtatgcggc tagccacagt aacgtagtgt ttccagacat gtcgggcacc gcactatcat 9120
gggtgcagaa aatctcgggt ggtctgtggg ccttcgcaat cggcgctatc ctggtgctgg 9180
```

FIG.6G

```
ttgtggtcac ttgcattggg ctccgcagat aagttagggt aggcaatggc attgatatag 9240
caagaaaatt gaaaacagaa aaagttaggg taagcaatgg catataacca taactgtata 9300
acttgtaaca aagcgcaaca agacctgcgc aattggcccc gtggtccgcc tcacggaaac 9360
tcgtggccaac tcatattgac acattaattg gcaataattg gaagcttaca taagcttaat 9420
tcggcgaata attggatttt tatttttatt tgcaattggt ttttaatatt tccaaaaaaa 9480
cgcacgtcca ctcggatggc taagggagat cctgaacttа acgctcgagt gccagccatc 9540
tgttgtttgc ccctcccccg tgccttcctt gaccctggaa ggtgccactc ccactgtcct 9600
ttcctaataa aatgaggaaa ttgcatcgca ttgtctgagt aggtgtcatt ctattctggg 9660
ggtgtggggtg gggcaggaca gcaaggggga ggattgggaa gacaatagca ggcatgctgg 9720
ggatgcggtg ggctctagga tctcgaccat gcagggtaag gatactgccc ggaacaaaac 9780
catgatcctg acgccatgcc agcctagttc taggtggagc tccagcttt gttcccttta 9840
gtgaggttta atttcgagct tggcgtaatc atggtcatag ctgtttcctg tgtgaaattg 9900
ttatccgctc acaatccac acaacatacg agccggaagc ataaagtgta aagcctgggg 9960
tgcctaatga gtgagctaac tcacattaat tgcgttgcgc tcactgcccg ctttccagtc 10020
gggaaacctg tcgtgccagc tgcattaatg aatcggccaa cgcgcgggga gaggcggttt 10080
gcgtattggg cgctcttccg cttcctcgct cactgactcg ctgcgctcgg tcgttcggct 10140
gcggcgagcg gtatcagctc actcaaaggc ggtaatacgg ttatccacag aatcagggga 10200
taacgcagga aagaacatgt gagcaaaagg ccagcaaaag gccaggaacc gtaaaaaggc 10260
cgcgttgctg gcgtttttcc ataggctccg ccccctgac gagcatcaca aaaatcgacg 10320
ctcaagtcag aggtggcgaa acccgacagg actataaaga taccaggcgt ttccccctgg 10380
aagctccctc gtgcgctctc ctgttccgac cctgccgctt accggatacc tgtccgcctt 10440
tctcccttcg ggaagcgtgg cgctttctca tagctcacgc tgtaggtatc tcagttcggt 10500
gtaggtcgtt cgctccaagc tgggctgtgt gcacgaaccc ccgttcagc ccgaccgctg 10560
cgccttatcc ggtaactatc gtcttgagtc caacccggta agacacgact tatcgccact 10620
```

FIG.6H

```
ggcagcagcc actggtaaca ggattagcag agcgaggtat gtaggcggtg ctacagagtt 10800
ctgaagtggg tggcctaact acggctacac tagaaggaca gtatttggta tctgcgctct 10860
gctgaagcca gttaccttcg gaaaaagagt tggtagctct tgatccggca aacaaccac 10920
cgctggtagc ggtggttttt ttgtttgcaa gcagcagatt acgcgcagaa aaaaggatc 10980
tcaagaagat ccttttgatct tttctacggg gtcttgacgct cagtggaacg aaaactcacg 11040
ttaagggatt ttggtcatga gattatcaaa aaggatcttc acctagatcc ttttaaatta 11100
aaaatgaagt tttaaatcaa tctaaagtat atatgagtaa acttggtctg acagttacca 11160
atgcttaatc agtgaggcac ctatctcagc gatctgtcta tttcgttcat ccatagttgc 11220
ctgactccgg gggggggggg cgctgaggtc tgcctcgtga agaaggtgtt gctgactcat 11280
accaggcctg aatcgcccca tcatccagcc agaaagtgag ggagccacgg ttgatgagag 11340
ctttgttgta ggtggaccag ttggtgattt tgaacttttg ctttgccacg gaacggtctg 11400
cgttgtcggg aagatgcgtg atctgatcct tcaactcagc aaaagttcga tttattcaac 11460
aaagccgccg tcccgtcaag tcagcgtaat gctctgccag tgttacaacc aattaaccaa 11520
ttctgattag aaaaactcat cgagcatcaa atgaaactgc aatttattca tatcaggatt 11580
atcaatacca tatttttgaa aaagccgttt ctgtaatgaa ggagaaaact caccgaggca 11640
gttccatagg atggcaagat cctggtatcg gtctgcgatt ccgactcgtc caacatcaat 11700
acaacctatt aatttcccct cgtcaaaaat aaggttatca agtgagaaat caccatgagt 11760
gacgactgaa tccggtgaga atggcaaaag cttatgcatt tctttccaga cttgttcaac 11820
aggccagcca ttacgctcgt catcaaaatc actcgcatca accaaaccgt tattcattcg 11880
tgattgcgcc tgagcgagac gaaatacgcg atcgctgtta aaaggacaat tacaaacagg 11940
aatcgaatgc aaccggcgca ggaacactgc cagcgcatca acaatatttt cacctgaatc 12000
aggatattct tctaatacct ggaatgctgt ttcccgggg atcgcagtgg tgagtaacca 12060
tgcatcatca ggagtacgga taaaatgctt gatggtcgga agaggcataa attccgtcag 12120
ccagtttagt ctgaccatct catctgtaac atcattggca acgctacctt tgccatgttt 12180
cagaaacaac tctggcgcat cgggcttccc atacaatcga tagattgtcg cacctgattg 12240
cccgacatta tcgcgagccc atttataccc atataaatca gcatccatgt tggaatttaa 12300
```

FIG.6I

```
tcgcggcctc gagcaagacg tttcccgttg aatatggctc ataacacccc ttgtattact 12360
gtttatgtaa gcagacagtt ttattgttca tgatgatata ttttttatctt gtgcaatgta 12420
acatcagaga tttgagaca caacgtggct ttcccccccc ccccgagct tgat          12474
```

CMV promoter 1 - 682
SFV replicon (before intron) 684 - 3678
Rabbit (-globin intron II 3679 - 4251
SFV replicon (after intron) 4252 - 9543
Hepatitis Delta virus ribozyme (antigenomic) 9544 - 9628
Kanamycin Gene 12342 - 11503
BamHI site for insertion of heterologous inserts 8677

27/39

Subcloning of the SFV replicon

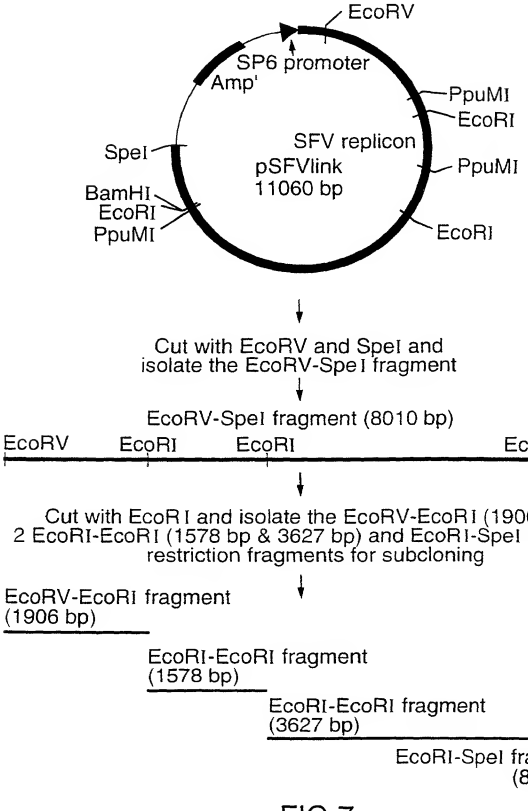

FIG.7

28/39

Construction of pMP76

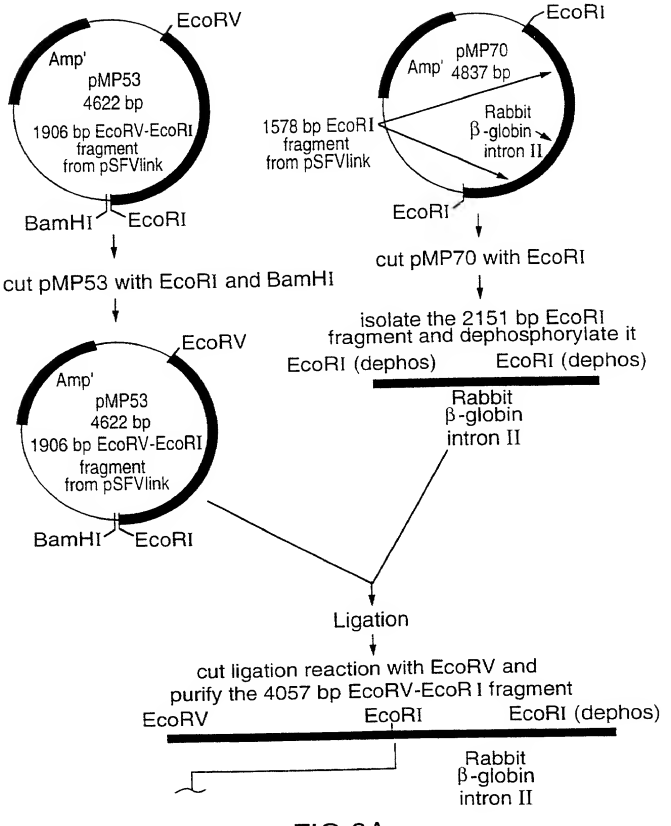

FIG.8A

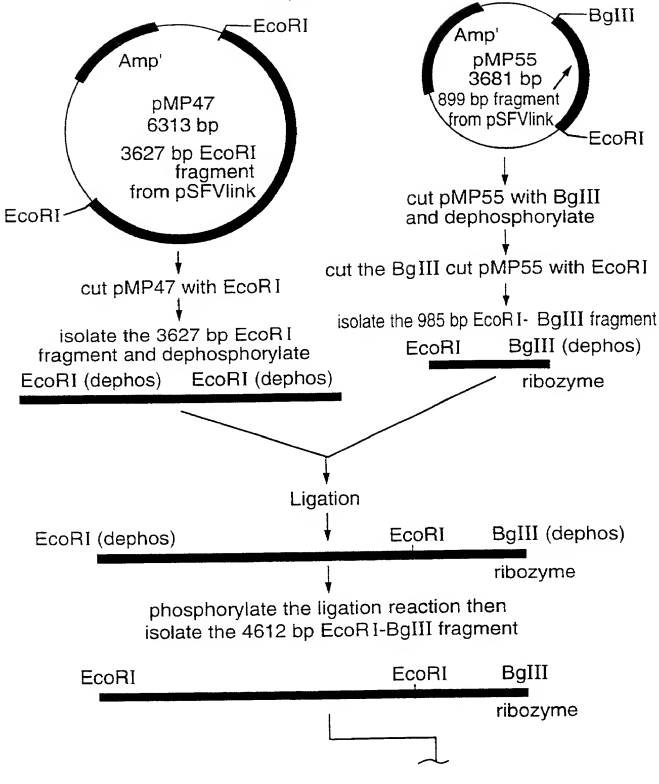
EcoRI
Amp'
pMP47
6313 bp
3627 bp EcoRI
fragment
from pSFVlink
EcoRI
cut pMP47 with EcoRI
isolate the 3627 bp EcoRI
fragment and dephosphorylate
EcoRI (dephos) EcoRI (dephos)
BgIII
Amp'
pMP55
3681 bp
899 bp fragment
from pSFVlink
EcoRI
cut pMP55 with BgIII
and dephosphorylate
cut the BgIII cut pMP55 with EcoRI
isolate the 985 bp EcoRI- BgIII fragment
EcoRI BgIII (dephos)
ribozyme
Ligation
EcoRI (dephos)
EcoRI
BgIII (dephos)
ribozyme
phosphorylate the ligation reaction then
isolate the 4612 bp EcoRI-BgIII fragment
EcoRI
EcoRI
BgIII
ribozyme

FIG.8B

30/39

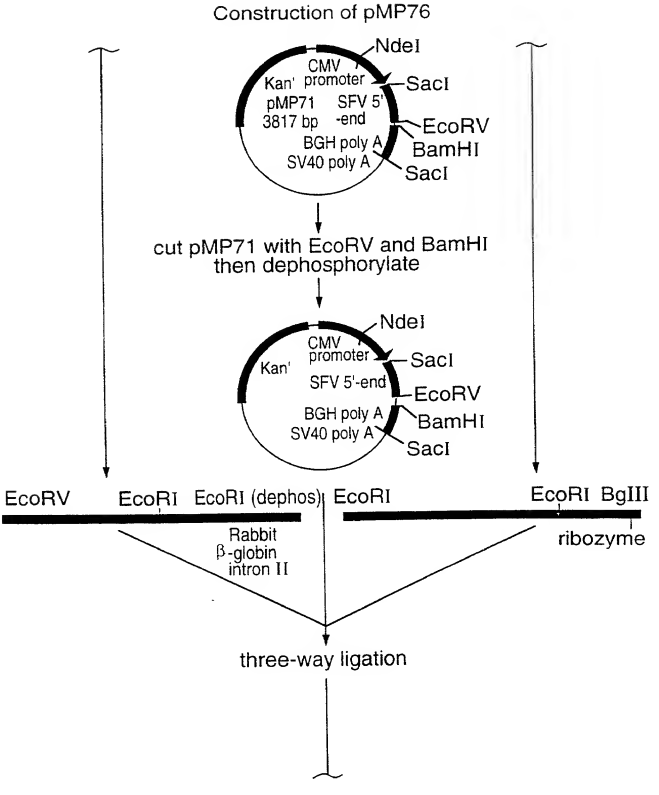
Construction of pMP76
NdeI
CMV promoter
Kan'
SacI
pMP71
3817 bp
SFV 5' -end
EcoRV
BamHI
BGH poly A
SV40 poly A
SacI
cut pMP71 with EcoRV and BamHI then dephosphorylate
NdeI
CMV promoter
Kan'
SacI
SFV 5'-end
EcoRV
BamHI
BGH poly A
SV40 poly A
SacI
EcoRV
EcoRI
EcoRI (dephos)
EcoRI
EcoRI BglII
Rabbit β-globin intron II
ribozyme
three-way ligation

FIG.8C

Construction of pMP76 (cont'd)

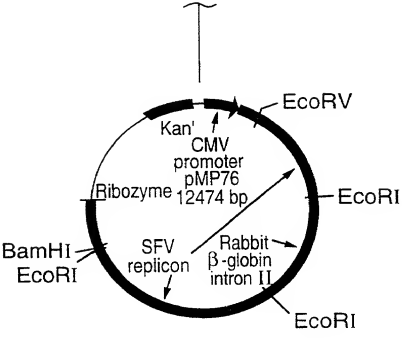

FIG.8D

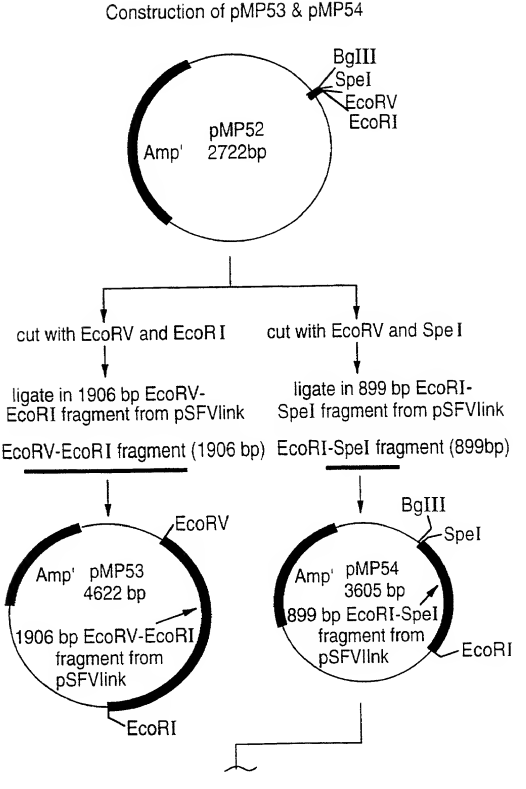
Construction of pMP53 & pMP54
BgIII
SpeI
EcoRV
EcoRI
pMP52
Amp' 2722bp
cut with EcoRV and EcoR I
cut with EcoRV and Spe I
ligate in 1906 bp EcoRV-EcoRI fragment from pSFVlink
ligate in 899 bp EcoRI-SpeI fragment from pSFVlink
EcoRV-EcoR I fragment (1906 bp)
EcoRI-SpeI fragment (899bp)
EcoRV
Amp' pMP53 4622 bp
1906 bp EcoRV-EcoRI fragment from pSFVlink
EcoRI
BgIII
SpeI
Amp' pMP54 3605 bp
899 bp EcoRI-SpeI fragment from pSFVIlnk
EcoRI

FIG.9A

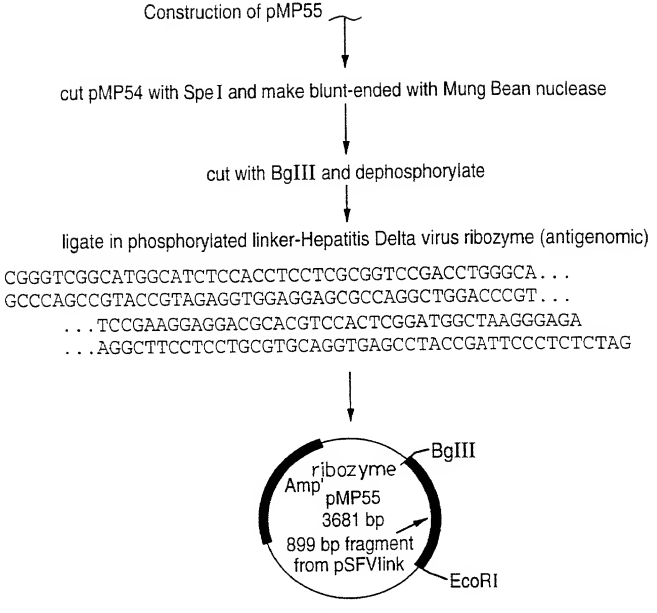
Construction of pMP55
cut pMP54 with Spe I and make blunt-ended with Mung Bean nuclease
cut with BgIII and dephosphorylate
ligate in phosphorylated linker-Hepatitis Delta virus ribozyme (antigenomic)
CGGGTCGGCATGGCATCTCCACCTCCTCGCGGTCCGACCTGGGCA...
GCCCAGCCGTACCGTAGAGGTGGAGGAGCGCCAGGCTGGACCCGT...
...TCCGAAGGAGGACGCACGTCCACTCGGATGGCTAAGGGAGA
...AGGCTTCCTCCTGCGTGCAGGTGAGCCTACCGATTCCCTCTCTAG
BgIII
ribozyme
Amp'
pMP55
3681 bp
899 bp fragment
from pSFVlink
EcoRI

FIG.9B

Construction of pMP52

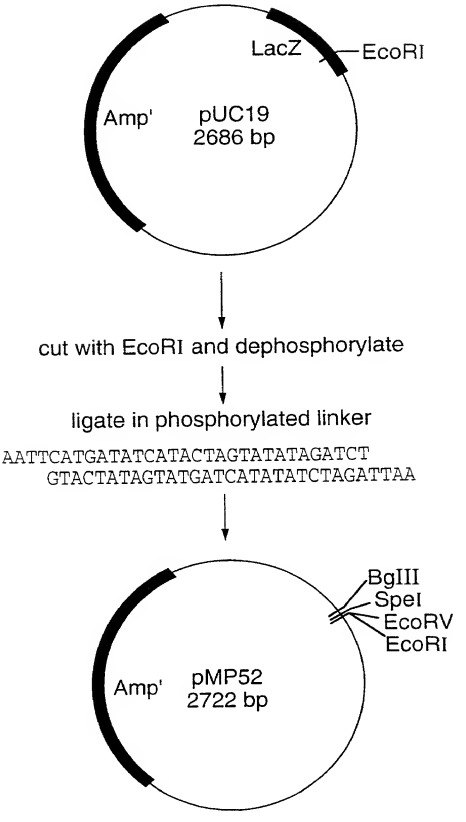

FIG.10

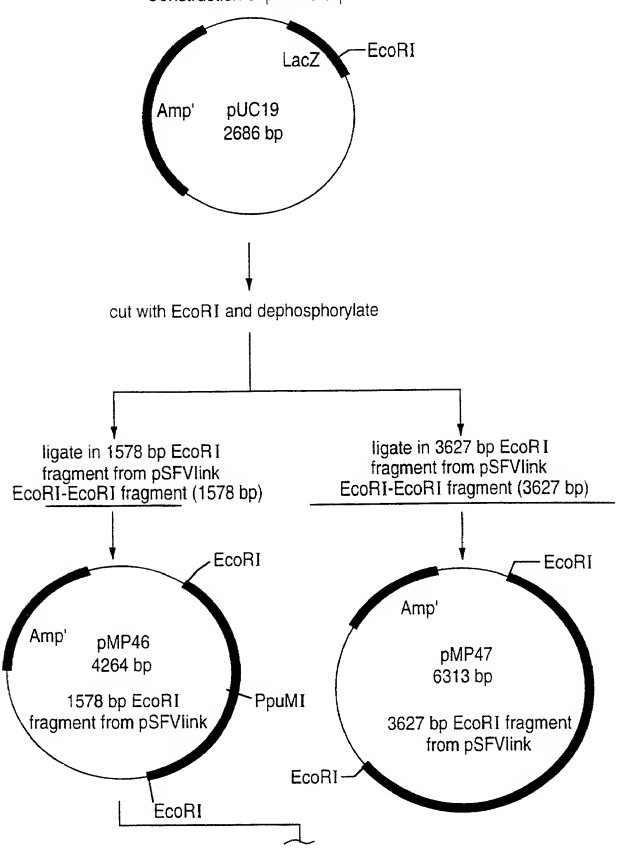
Construction of pMP46 & pMP47
LacZ
EcoRI
Amp'
pUC19
2686 bp
cut with EcoRI and dephosphorylate
ligate in 1578 bp EcoRI fragment from pSFVlink EcoRI-EcoRI fragment (1578 bp)
ligate in 3627 bp EcoRI fragment from pSFVlink EcoRI-EcoRI fragment (3627 bp)
EcoRI
Amp'
pMP46
4264 bp
1578 bp EcoRI fragment from pSFVlink
PpuMI
EcoRI
EcoRI
Amp'
pMP47
6313 bp
3627 bp EcoRI fragment from pSFVlink
EcoRI

FIG.11A

Construction of pMP70

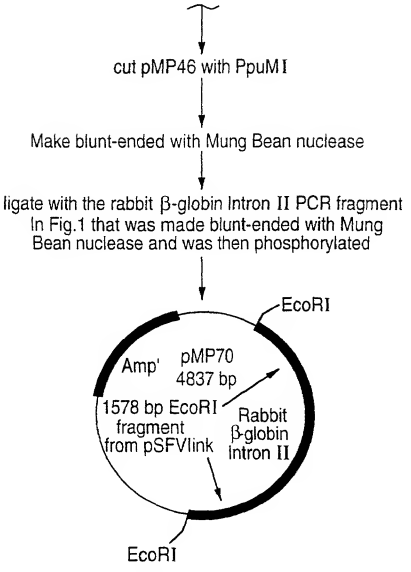

FIG.11B

37/39
Construction of pMP71

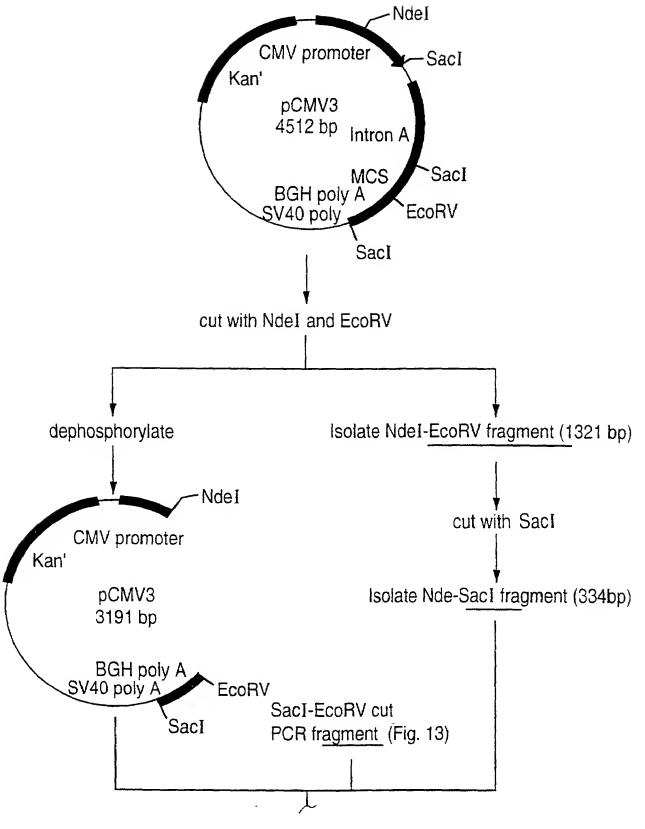

FIG.12A

Construction of pMP71 (cont'd)

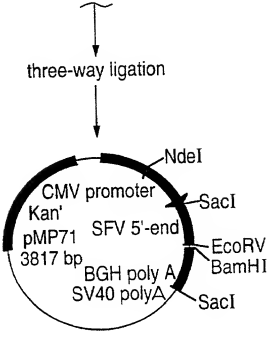

FIG.12B

FIG.13

```
1   CGTTTAGTGA ACCGTATGGC GGATGTGTGA CATACACGAC GCCAAAAGAT 50

51  TTTGTTCCAG CTCCTGCCAC CTCCGCTACG CGAGAGATTA ACCACCCACG 100

101 ATGGCCGCCA AAGTGCATGT TGATATTGAG GCTGACAGCC CATTCATCAA 150

151 GTCTTTGCAG AAGGCATTTC CGTCGTTCGA GGTGGAGTCA TTGCAGGTCA 200

201 CACCAAATGA CCATGCAAAT GCCAGAGCAT TTTCGCACCT GGCTACCAAA 250

251 TTGATCGAGC AGGAGACTGA CAAAGACACA CTCATCTTGG AT      292
```

Docket No.
1038-1030 MIS:jb

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next ~~to my~~ name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

ALPHAVIRUS VECTORS

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on **November 13, 1998** as ~~United States Application No.~~ or PCT International Application Number **PCT/CA98/01065**

and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

Michael I. Stewart (24,973)

Send Correspondence to: **Sim &McBurney**
6th Floor, 330 University Avenue
Toronto, Ontario
Canada, M5G 1R7.

Direct Telephone Calls to: *(name and telephone number)*
(416) 595-1155

1-00

Full name of sole or first inventor
Mark Parrington

Sole or first inventor's signature — Mark Parrington — Date: June 12, 2000

Residence
Bradford, Ontario, Canada CAX

Citizenship
Canadian

Post Office Address
45 Martin Street, Bradford, Ontario, Canada, L3Z 1Z4.

2-00

Full name of second inventor, if any
Michel H. Klein

Second inventor's signature — Date: July 25, 2000

Residence
Toronto, Ontario, Canada CAX

Citizenship
Canadian

Post Office Address
16 Munro Boulevard, Toronto, Ontario, Canada, M2P 1B9.

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

60/065,793	November 14, 1997
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/CA98/01065	November 13, 1998	
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.